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New insights into using lipid based suspensions for ‘brick dust’ molecules: case study of Nilotinib

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Running head:

Lipid suspensions for Nilotinib

Abstract

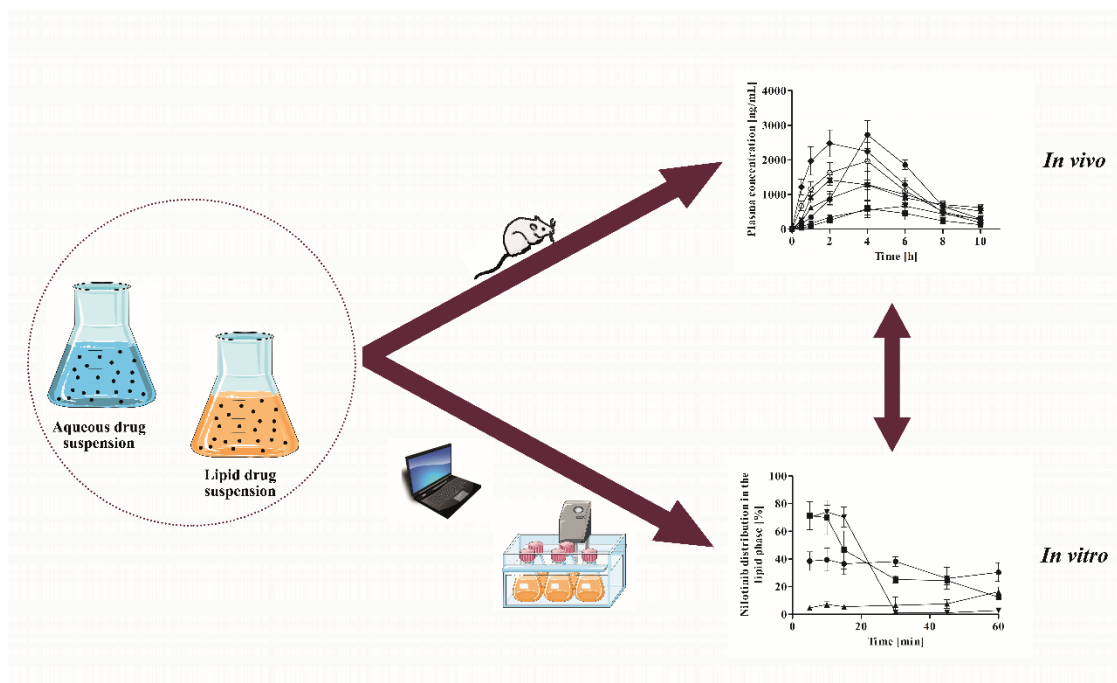
Purpose Lipid suspensions have been shown to be a suitable bio-enabling formulation approach for highly lipophilic or ‘grease ball’ drug molecules, but studies on ‘brick dust’ drugs are lacking. This study explored the utility of lipid suspensions for enhancing oral bioavailability of the rather hydrophobic drug nilotinib *in vivo* in rats.

Methods Four lipid suspensions were developed containing long chain triglycerides, medium chain triglyceride, long chain monoglycerides and medium chain monoglycerides and *in vivo* bioavailability was compared to an aqueous suspension. Additionally, *in vitro* lipolysis and wettability tests were conducted.

Results Nilotinib lipid suspensions did not show a bioavailability increase compared to an aqueous suspension. The bioavailability was lower for triglyceride suspensions, relative to both monoglyceride and an aqueous suspension. The long chain monoglyceride displayed a significantly higher bioavailability relative to triglycerides. *In vitro* lipolysis results suggested entrapment of nilotinib crystals within poorly dispersible triglycerides, leading to slower nilotinib release and absorption. This was further supported by higher wettability of nilotinib by lipids.

Conclusion Monoglycerides improved oral bioavailability of nilotinib in rats, relative to triglycerides. For ‘brick dust’ drugs formulated as lipid suspensions, poorly dispersible formulations may delay the release of drug crystals from the formulation leading to reduced absorption.

Graphical abstract



Graph. Abstract: An aqueous and four lipid suspensions have been evaluated *in vitro* and *in vivo* to gain insights into the potential benefits and limitations of lipid suspensions.

Keywords Lipid suspension, Lipid based formulation, Brick dust, Nilotinib, Bio-enabling formulation

1 Introduction

In recent years, there has been an emerging trend towards the discovery of drug candidates that display sub-optimal developability characteristics (1). A key shift is the increasing number of lead drug candidates displaying poor aqueous solubility, where it is estimated that up to 75% of drugs in development are classified as Class II/IV in the biopharmaceutical classification system (BCS) (2). There is a general increase in molecular weight (2, 3) as well as lipophilicity (4), with the intention to improve target receptor selectivity and maximise potency. These highly lipophilic drug candidates may consequently display solubility limitations and hence require bio-enabling formulation approaches such as nanosizing or lipid based formulations (LBF) to ensure sufficient oral absorption. In particular, LBFs have demonstrated commercial potential for delivery of drugs with high lipophilicity. For such so-called ‘grease ball’ drug candidates, LBFs are considered favourable to increase drug solubilization in the intestinal tract and, in general, good dose loading capacity can be achieved within lipid vehicles. It has been suggested that a drug with a $\log P$ of > 4 would be best to achieve adequate solubility in pure triglycerides (TG), while an intermediate $\log P$, between 2 and 4, may result in a suitable solubility in mixtures of lipids including mono-, di- and triglycerides, hydrophilic surfactants and water-soluble co-solvents, dependent on the dose (5).

While poor aqueous solubility driven by high lipophilicity provides good drug candidates for LBFs, the situation is more complex in the case of drugs displaying high hydrophobicity (6). Such high melting drug candidates are often formulated using amorphous solid dispersions to diminish the impact of the solid state on the dissolution (7). Thus, the forces within the crystal lattice can also be a key determinant for the suitability of a drug using LBF, as these must be overcome prior to drug solvation in the LBF. Therefore, for molecules that display ‘brick dust’

characteristics, dose loading in LBFs is limited by the high crystal lattice energy. It has been reported that compounds with a melting point (T_m) above 150 °C display poor solubility in triglycerides (8), theoretically limiting the classical approach of lipid solutions to low hydrophobic and high lipophilic molecules. While such T_m and $\log P$ based guides are helpful, it should also be kept in mind that the majority of drugs emerging from drug discovery display melting points > 150 °C and $\text{clog}P > 2$. For example, a recent study on the melting point distribution of globally available drugs suggests that > 61% of drugs have a T_m higher than 150 °C and $\text{clog}P > 2$ (9). Nevertheless, numerous drugs with a $T_m > 150$ °C have benefited from LBFs and more recent lipid-based formulation approaches have the potential to overcome dose-loading limitations, such as super-SNEDDS (6, 10, 11), ionic liquids (12, 13) and lipid suspensions (6, 14).

Lipid suspensions, where crystalline drug is dispersed in a lipid vehicle within an oral capsule, offer a scalable approach for oral administration, with the potential to enhance oral absorption via excipient-mediated effects on solubilisation within the intestine. Additionally, the excipients in lipid suspensions may offer the benefit of increased intestinal permeability and/or promotion of intestinal lymphatic transport (15, 16). Lipid suspensions offer the potential benefit for sustained delivery via particle size mediated control of the dissolution rate of the suspended drug particles. In general, lipid suspensions may be particularly useful in a preclinical setting for poor soluble drug candidates, where high dosing in rodent models is necessary for early stage toxicological evaluation (17).

Lipid suspensions have been investigated with different excipients and drugs for their benefit *in vivo* with the general experience that in most performed studies beneficial effects have been observed. Drugs such as griseofulvin (T_m 220 °C, $\log P$ 2.2), atovaquone (T_m 216 - 219 °C, $\log P$

5.8) (18), phenytoin (T_m 295 °C, $\log P$ 2.5), diacerein (T_m 217 °C, $\log P$ 2.0), danazol (T_m 227 °C, $\log P$ 4.9), or fenofibrate (T_m 79 °C, $\log P$ 5.1) have been investigated (19-24). For example, a griseofulvin corn oil suspension resulted in a higher bioavailability compared to an aqueous suspension, when dosed orally to rats (25). In the case of danazol and fenofibrate, administration of lipid suspensions, by reducing the amount of lipid excipient in the formulation, resulted in similar bioavailability to the lipid solutions (21, 22).

In terms of ‘brick dust’ molecules with a high hydrophobicity as well as lipophilicity similar to the used model drug in this study, there are limited reports in the literature exploring the utility of lipid suspensions. Danazol showed an 4-9-fold increase in bioavailability in rats using a Labrafil M2125CS suspension compared to an aqueous suspension. Furthermore, one of the tested Labrafil suspensions showed equivalent exposure to the Labrafil solution (21). Roland *et al.* employed a lipid suspension approach for atovaquone, a potent antiprotozoal drug (24). The bioavailability of atovaquone is 3.3-fold higher after a high fat meal, however, the drug displays limited solubility in medium chain triglycerides (~4 mg/mL). In *in vivo* studies in humans, atovaquone bioavailability was similar for a lipid suspension (500 mg in 30 mL medium chain triglycerides) and an aqueous suspension (500 mg in 30 mL of 0.25% methyl cellulose solution). Moreover, the lipid suspension absorption was prolonged as evident by longer t_{max} and lower c_{max} compared to the aqueous suspension (24). Thus, the potential benefit of lipid suspensions for highly lipophilic and hydrophobic drugs is not clear and merits further investigation.

Nilotinib (Figure 1) is a tyrosine kinase inhibitor which was approved for the treatment of chronic myelogenous leukemia in 2007. Nilotinib displays high lipophilicity ($\log P$ ~5) and

higher bioavailability after ingestion of a high fat meal (> 80%), which are both considered favourable characteristics from a LBF perspective. However, the T_m of nilotinib is 236 °C hence the expected solubility in lipids is solid-state limited. Therefore, nilotinib was chosen as a model 'brick dust' drug for the present study, where the aim was to investigate the potential benefit of a lipid suspension as formulation approach. The *in vivo* bioavailability of a series of lipid suspensions was compared to an aqueous suspension. In addition, the *in vitro* lipolysis model was employed to provide mechanistic insights on the formulation performance.

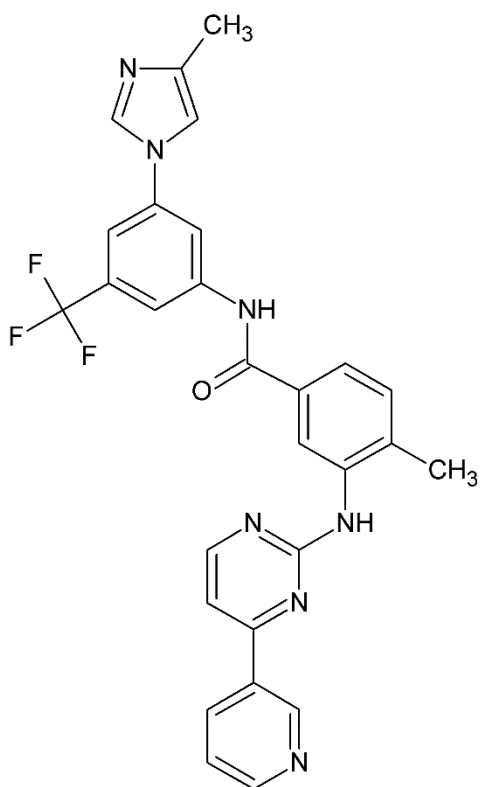


Figure 1 Chemical structure of nilotinib

2 Materials and methods

2.1 Chemicals and materials

Nilotinib and sorafenib were purchased from Kemprotec Ltd. (UK). Olive oil (LC TG), highly refined and low acidity, capric acid, L- α -phosphatidylcholine Type XI-E (PC) (768 g/mol), taurodeoxycholic acid (NaTDC) and pancreatic lipase (8 x USP) were obtained from Sigma-Aldrich (Ireland). Capmul MCM[®] (MC MG) and Captex 1000[®] (MC TG) were kindly donated by Abitec corporation (USA). Monocaprin was obtained from TCI Germany and oleic acid was received from VWR (Ireland). A sample of Peceol[®] (LC MG) was kindly donated by Gattefossé (France) and SIF powder version 1 was kindly donated by biorelevant.com (UK). All other chemicals and solvents were of analytical or HPLC grade and were purchased from Sigma-Aldrich (Ireland) and used as received.

2.2 Particle size measurements

Wet laser diffraction analysis was performed using a Mastersizer 3000 (Malvern Instruments Limited, United Kingdom), equipped with a Hydro MV medium automated dispersion unit with a 120 mL dispersant volume. Nilotinib sample solution was prepared by adding excess nilotinib to HPLC grade water. The suspension was ultrasonicated for 5 sec. before the measurement. A refractive index of 1.4 was used for water as a reference index for statistical calculation using the particle sizing program. A refractive index value of 1.65 (26), absorption index of 0.1 and density of 1.362 g/cm³ were used for particle size distribution analysis of nilotinib. The nilotinib sample was added drop-wise into the saturated wet dispersion unit containing approximately 100 mL of dispersant (water) until obscuration reached between 1.2 % and 5.4 %, at a stirring speed of 1250 rpm. D10, D50, D90 are reported for all the samples, where n=3. The results of the laser diffraction analysis were confirmed by optical

microscopy using an Olympus BX51 equipped with an Olympus BC 100 camera. Measurements were done at 40 x magnification with Olympus Stream Start version 1.7.

2.3 Solubility studies

Equilibrium solubility was determined in olive oil, Captex 1000, Peceol and Capmul MCM using the shake flask method. In brief, an excess of nilotinib was added to the excipients, thoroughly mixed and shaken in a water bath shaker at 37 °C (n=3). Samples were taken after 24 h, 48 h, 72 h and centrifuged at 21,380 x g (Mikro 200 R, Hettich GmbH, Germany) and 37 °C for 15 min. The supernatant was transferred to a new tube and centrifuged again under identical conditions. In order to solubilise the oily excipient, the supernatant was diluted approximately 1:5 - 1:50 with a mixture of tetrahydrofuran (THF) and dimethylformamide (DMF) (50:50), followed by further dilution with DMF and dimethyl sulfoxide (DMSO). The obtained samples were analysed by reverse phase HPLC, as described below. Equilibrium was assumed once two time-points had a variation of less than 10 %. All samples were run in triplicates.

2.3.1 Biorelevant solubility and dispersion

Fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF) were prepared according to the instructions by biorelevant.com. FeSSIF was used directly, whereas FaSSIF was left at room temperature for 2 hours prior further usage.

Nilotinib's equilibrium solubility in a biorelevant dispersion of the lipid formulation was simulated by adding 2 g of olive oil (FaSSIF_{LC}) or Captex 1000 (FaSSIF_{MC}) to 80 mL of prepared FaSSIF. The mixture was stirred at 37 °C for 40 min prior to the addition of excess nilotinib.

184

185 The post digestion equilibrium solubility of the triglyceride formulations was simulated by
186 adding the expected lipolysis components to FaSSIF media, similar to the artificial digestion
187 media suggested by Gautschi and co-workers (27). The measured equilibrium solubility
188 resembled the maximum solubility increase upon complete digestion of the TG excipients.
189 Oleic acid and α -monooleat (FaSSIF_{LCdig}) or capric acid and α -monocaprin (FaSSIF_{MCdig}) in
190 a molar ratio of 2:1 were added to FaSSIF in order to simulate the digestion of long chain or
191 medium chain triglycerides, respectively. Where necessary, the excipients were molten first
192 and mixed thoroughly before 2 g of this mixture was added to 80 mL of medium. The dispersion
193 was stirred at 37 °C for 40 min and the pH was adjusted to 7.5 prior to the addition of excess
194 nilotinib.

195

196 After the addition of excess nilotinib all samples were placed in a water bath shaker at 37 °C.
197 After 3 h, 6 h and 24 h samples were taken and analysed. All taken samples were processed
198 like the lipid solubility samples. The resulting supernatant was diluted with a mixture of THF,
199 DMF and DMSO (1.25:23.75:75) before analysis.

200

201 The samples were analysed using an Agilent 1200 series HPLC system comprising a binary
202 pump, degasser, autosampler and variable wavelength detector. Data analysis was done with
203 EZChrom Elite version 3.2. In order to separate the lipids from nilotinib a Zorbax Eclipse Plus-
204 C18 column (5 μ m, 4.6 mm x 150 mm) with a Zorbax Eclipse Plus-C18 guard column (5 μ m,
205 4.6 mm x 12.5 mm) was used. The mobile phase consisted of 20 mM Phosphate buffer pH 2
206 and methanol (53:47) and was used at a flow rate of 1 mL/min. The column temperature was
207 set to 25 °C and the detection wavelength was 255 nm. The lower limit of quantification for
208 this method was 25 ng/mL.

209

210 2.4 *In Vitro* Evaluation: Drug Solubilization during Formulation Dispersion and 211 Digestion

212 *In vitro* lipolysis was performed using a pH-stat apparatus (Metrohm AG, Herisau,
213 Switzerland) comprising a Titrand 907 stirrer, 804 Ti-stand, a pH electrode (Metrohm) and
214 two 800 Dosino dosing units coupled to a 20 mL autoburette. The system was operated by the
215 Tiamo 2.2 software. The *in vitro* protocol was amended from Williams *et al.* (28, 29) except
216 that the overall volume of the buffer was increased to allow for a higher sample yield. The ratio
217 of formulation (1.583g) to digestion buffer (57ml) remained constant. In brief, the buffer
218 contained 2 mM TRIS maleate, 150 mM NaCl, 1.4 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, adjusted to pH 7.5. For
219 the digestion experiments the buffer was supplemented with 3 mM NaTDC and 0.75 mM PC
220 (digestion buffer) and stirred for 12 hours before further usage. The pancreatin extract was
221 prepared freshly by adding 5 mL of 5 °C buffer to 1 g of porcine pancreatic enzymes (8x USP),
222 which was vortexed thoroughly. The mixture was centrifuged for 15 min at 5 °C, 2800 g
223 (Hettich Rotina 380R) and 4 mL of supernatant were recovered and stored at 2 – 8 °C before
224 further usage.

225

226 For the *in vitro* lipolysis experiment 1.583 g of suspension (10 mg/mL) was dispersed into 57
227 mL of digestion buffer for 10 min. Three 1 mL samples were taken at 2.5, 5 and 10 min from
228 the middle of the vessel. pH of the media was adjusted and maintained at 7.5 throughout
229 digestion using the pH stat method of the Titrand device with 0.2 M NaOH and 0.6 M NaOH
230 for long and medium chain formulations, respectively. The amount of dispensed NaOH was
231 recorded by the system. To the remaining 54 mL (1.5 g lipid formulation) dispersion, 6 mL of

pancreatic extract was added to initialize digestion. After 60 min the released non-ionized free fatty acids were determined by a pH increase of the buffer to pH 9.

Samples of 4.9 mL were taken at 5, 10, 15, 30, 45 and 60 min during the digestion experiment from the middle of the vessel. In each sample and after 60 min the enzymes were inhibited by the addition of 1 M 4- Bromophenylboronic acid in methanol (5 μ L per mL sample). All samples containing a lipid phase were centrifuged at 37 °C and 400,000 g for 30 min (Beckman Coulter Optima L-90K, Rotor: VTI 65.2). Samples, that did not contain a lipid phase (aqueous suspension) were centrifuged at 37 °C and 21,000 g for 30 min using a benchtop centrifuge (Hettich Micro 200R).

2.5 Contact angle measurements

Nilotinib's wettability was determined using the contact angle measurement by the sessile drop technique. Nilotinib disks were prepared according to Muster *et al.* (30). In brief, 40 mg nilotinib were compacted for 1 min with a pressure of approximately 210 MPa (Star Specac manual hydraulic press). 6 μ L of Peceol, Capmul MCM, olive oil, Captex 1000, 0.5 % (w/v) methylcellulose in water and pure water, respectively, were placed on the pressed disk using a fully automated optical tensiometer (Theta Attension by Biolin Scientific). After the drop was released the contact angle was captured using 76 frames per sec (FPS) for 20 sec followed by 7.6 FPS for 100 sec. The contact angle was calculated directly, 0.5 sec, 60 sec and 120 sec after the drop release using the fit of the droplet's shape to the Young-Laplace equation. The contact angle for one measurement was the mean of the individual calculated angles of each side of the droplet. All measurements were done on 3 disks and consisted of at least 5 measurements per time point.

2.6 Formulations for *in vivo* and *in vitro* studies

The lipid formulations were prepared by combining 10 mg nilotinib with 1 mL lipid excipient followed by an overnight stir prior to dosing. The aqueous formulation was prepared by adding 10 mg of nilotinib to 1 mL of the aqueous 0.5 % (w/v) methylcellulose solution and mixed thoroughly. In order to decrease the powder agglomerates the suspension was placed in an ultrasonic bath for 5 sec and vortexed again afterwards.

2.7 *In vivo* study

The protocol used for the *in vivo* pharmacokinetic study was approved by the institutional animal ethics committee in accordance with Belgian law regulating experiments on animals and in compliance with EC directive 2010/63/EU and the NIH guidelines on animal welfare. Male Sprague-Dawley rats weighing 280-320 g on the day of the experiments were purchased from Charles River Laboratories Deutschland (Sulzfeld, Germany) and maintained on standard food and water ad libitum in the laboratory for at least 5 days before entering the experiment. For the fasted study legs food was removed 16-20 h before dosing and water was available ad libitum at all times. In the case of the fed study leg, food was available throughout the study and was not removed. Parallel groups of animals were administered with each formulation at a volume of 2 mL/kg by oral gavage with a nilotinib dose of 20 mg/kg. By individual tail vein puncture, 200 μ L blood samples were collected into plasma collection tubes containing dipotassium EDTA. Samples were taken at 0.5, 1, 2, 4, 6, 8, 10 and 24 h following oral dosing. Plasma was harvested immediately by centrifugation for 10 min at $1,000 \times g$ and stored at -80 °C until analysis. After the experiment the animals were euthanized.

2.8 Bioanalysis

The plasma concentrations of nilotinib were determined by reversed phase ultra-performance liquid chromatography (UPLC). The Agilent 1260 series UPLC system comprised a binary pump, degasser, temperature controlled autosampler, column oven and diode array detector. The system was controlled, and the data analysed with EZChrom Elite version 3.3.2. The used method was modified from Pirro *et al.* (31). In brief, a Zorbax Eclipse Plus-C18 column (5 µm, 4.6 mm x 150 mm) with a Zorbax Eclipse Plus-C18 guard column (5 µm, 4.6 mm x 12.5 mm) was used. The mobile phase consisted of water, methanol, acetonitrile and triethylamine (34:30:35:1 v/v) and was used at a flow rate of 0.9 mL/min. The sample and column temperature were set at 5 °C and 25 °C, respectively, and the detection wavelength was 267 nm. Nilotinib was extracted from the plasma samples by liquid-liquid extraction. To 50 µL of the plasma sample 66 µL of a methanol acetonitrile mixture (30:35 v/v), containing 1.25 µg/mL sorafenib as internal standard, was added. The mixture was mixed thoroughly and centrifuged at 22 °C, 11,500 x g for 9 min. 50 µL of the supernatant was injected to the UPLC system for analysis. The limit of quantification in plasma by this method was 10 ng/mL and linearity was confirmed between 10 ng/mL and 4 µg/mL. The extraction efficiency was found to be > 92.5 % across the concentration range and the intra- and inter-day variability was 4.2 % and 5.4 % at maximum, respectively.

2.9 Data Analysis

After using the Bartlett's test to check for equal variance a one-way ANOVA was performed for the lipolysis data using a Tukey post-hoc test to compare the different formulation performances. The solubility limited absorption dose (SLAD) was calculated for the biorelevant media and dispersions according to the following equation (1):

303
$$SLAD = S_{Si} \times V \times M_p$$

304 where S_{Si} is the solubility in the different media, V the fluid volume available in the intestine
305 (500 mL) and M_p is the permeability dependent multiplier, which for low permeable drugs like
306 nilotinib was kept at unity.

307

308 The pharmacokinetic parameters were calculated using Microsoft Excel. The plasma
309 concentration profiles were analysed by non-compartmental analysis and calculation of each
310 area under the curve (AUC) was based on the linear trapezoidal rule. Mean residence time
311 (MRT) was calculated according to the following equation:

312
$$MRT = \frac{AUMC_{0-inf}}{AUC_{0-inf}}$$

313 where $AUMC_{0-inf}$ is the area under the first moment curve from timepoint 0 to infinity and
314 AUC_{0-inf} is the area under the curve from timepoint 0 to infinity.

315

316 The statistical analysis for all *in vivo* parameters was performed using a one-way analysis of
317 variance (one-way ANOVA) after using the Bartlett's test to check for equal variance. The
318 Gaussian distribution of the data was tested with the Kolmogorov-Smirnov test and the
319 pairwise comparison of the groups was done using Tukey's multiple comparison test. All
320 statistical analyses were carried out using GraphPad Prism 5.

321

Table I Physicochemical properties and in vivo behaviour of nilotinib.

Literature data	
Log <i>P</i> (32)	4.95
Molecular weight [g/mol] (33)	565.98
pKa values (33)	2.1, 5.4
BCS class (34)	II/IV
DCS class	IIb/IV
Food effect (33)	29 % AUC increase with a light meal 82 % AUC increase with a high fat meal
Experimental data	
Melting point [°C] (by DSC)	236.42 ± 0.22
FaSSIF solubility [µg/mL]	0.32 ± 0.03
FeSSIF solubility [µg/mL]	3.16 ± 0.09
<i>In vitro</i> FeSSIF/FaSSIF ratio	9.98
Olive oil solubility [µg/mL]	6.82 ± 0.45
Peceol solubility [µg/mL]	928.76 ± 35.24
Captex 1000 solubility [µg/mL]	50.19 ± 8.15
Capmul MCM solubility [µg/mL]	3361.21 ± 318.01
Employed particle size [µm]	1.31 ± 0.40 (D10) 7.41 ± 4.06 (D50) 197.88 ± 22.81 (D90)

3 Results

3.1 Solubility in lipid excipients

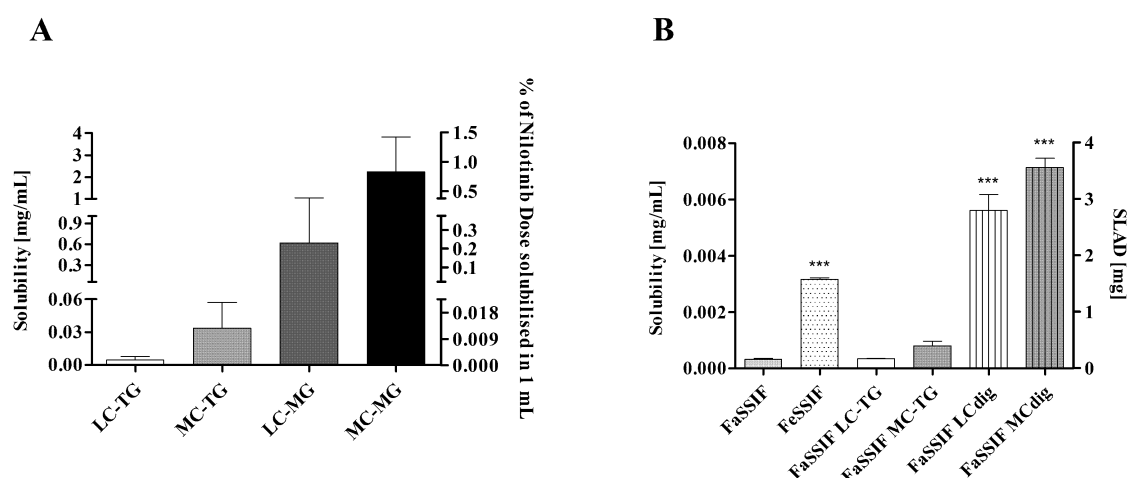


Figure 2 A: Nilotinib equilibrium solubility in LC-TG (olive oil), MC-TG (Captex 1000), LC-MG (Peceol), MC-MG (Capmul MCM) (n=3) and the % of a 300 mg Dose solubilised in 1 mL of lipid excipient at 37 °C. **B:** Nilotinib solubility in FaSSIF, FeSSIF and biorelevant lipid dispersions (n ≥ 3) and the amount of drug that can be dissolved in 500 mL of a biorelevant lipid dispersion utilizing the solubility limited absorption dose (SLAD).

Nilotinib is a high T_m and high $\log P$ compound, hence displaying properties of a ‘brick dust’ molecule (Table I, Figure 1). Initial solubility screening in pure lipid excipient indicated that nilotinib was practically insoluble (35) in LC and MC TGs (Figure 2 A). The solubility was higher in monoglycerides (MG) compared to TG lipids. Within the TGs and MGs, a higher solubility was observed for the MC compared to the LC excipients. Overall, the percent of the therapeutic dose (300 mg) that would be dissolved in 1 mL lipid ranged between 0.01-1.5 % (Figure 2 A). This confirms that despite a high $\log P$ for nilotinib, the use of a classical lipid solution approach was not feasible, and hence lipid suspensions were developed to evaluate if lipids could still have a bio-enhancing influence on nilotinib.

Subsequently, nilotinib solubility was determined under biorelevant conditions. Solubility in FaSSIF was low at 0.0001 % of a 300 mg dose, whereas it increased approximately 10-fold in simulated fed state media (Figure 2 B, Table I). Indeed, nilotinib's bioavailability is reported to be higher in the fed state (82 % increase in AUC after a high fat meal). Moreover, solubility was screened in lipidic dispersions to subsequently assess the nilotinib solubility on aqueous dispersion of lipid formulations in biorelevant media. Overall the solubility increases in the pure TG lipid dispersions (FaSSIF_{LC TG} and FaSSIF_{MC TG}) were relatively low, whereas lipid excipients that simulate post-digestive intestinal conditions (FaSSIF_{LCdig} and FaSSIF_{MC dig}) suggested significantly higher solubilisation capacity for nilotinib. The post digestive media showed an increase in the solubility limited absorption dose (SLAD) from 0.16 in FaSSIF to 2.81 and 3.57 for FaSSIF_{LCdig} and FaSSIF_{MCdig}, respectively. Despite this increase in SLAD, the overall SLAD obtained was substantially lower than the therapeutic dose.

3.2 *In vivo* bioavailability of nilotinib

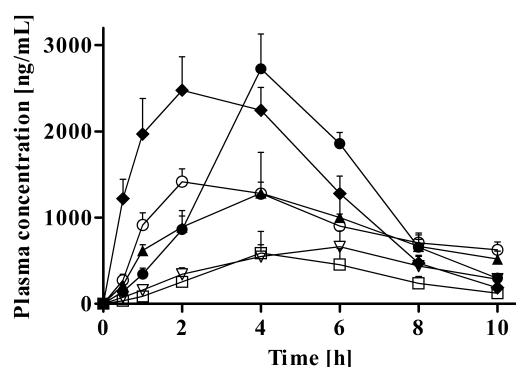


Figure 3 Plasma concentration profiles as a function of time (Mean \pm SEM for n=5) for a crude aqueous suspension in the fasted state (◆), crude aqueous suspension in the fed state (○), LC MG [Peceol] (●), LC TG [olive oil] (□), MC MG [Capmul MCM] (▲) and MC TG [Captex 1000] (▽) suspension in male sprague-dawley rats.

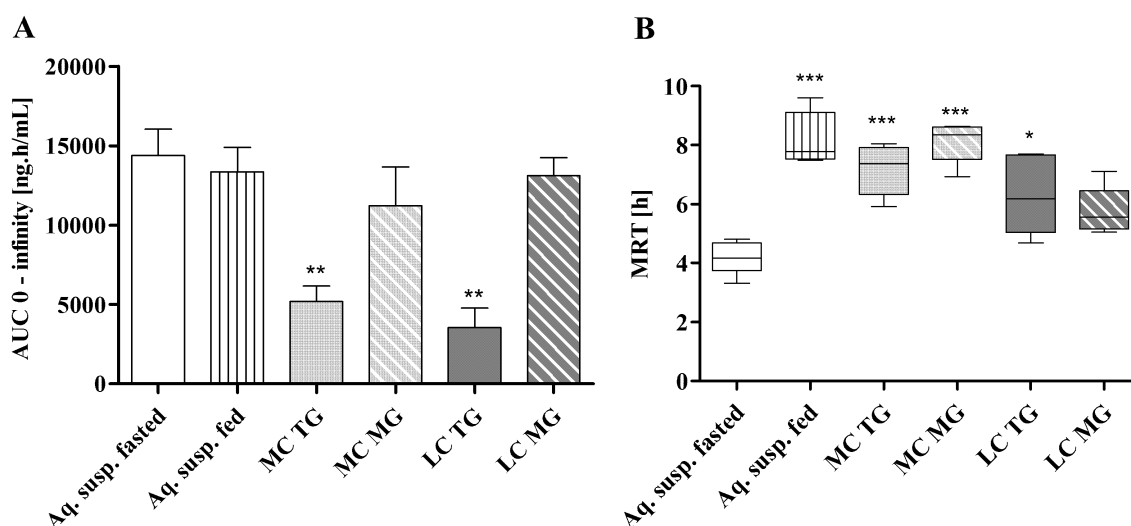


Figure 4 Results of the *in vivo* study of nilotinib dosed in an aqueous suspension in the fasted and fed state, a MC TG (Captex 1000), MC MG (Capmul MCM), LC TG (olive oil) and LC MG (Peceol) suspension to male sprague-dawley rats (n=5) The given statistical significance was compared to the aqueous suspension in the fasted state. A: AUC 0 h - infinity (mean \pm SEM) and B: Mean residence time (MRT) (Whiskers: min. to max. value)

Nilotinib suspensions were prepared in olive oil (LC TG), Captex 1000 (MC TG), Peceol (LC MG) and Capmul MCM (MC MG) and bioavailability was assessed *in vivo* in rats. The dose and lipid amount were fixed at 20 mg/kg and 2 mL/kg, respectively. The amount of nilotinib present in these lipid suspensions exceeded the equilibrium solubility in the lipid vehicles 1471-fold for the LC TG formulation, 199-fold for the MC TG formulation, 11-fold for the LC MG formulation and 3-fold for the MC MG formulation. An aqueous nilotinib suspension was dosed as a comparator, and additionally nilotinib's food effect was investigated by the administration of an aqueous suspension in the fed state. The mean plasma concentration versus time profiles are presented in Figure 3 and the AUC from 0 hours to infinity and mean residence time (MRT) for nilotinib after oral administration of the lipid and aqueous suspensions are shown in Figure 4. Table II presents a summary of the pharmacokinetic parameters obtained.

Among the lipid formulations, the performance ranking of the LBF suspensions showed that the highest exposure was achieved for Peceol (LC MG) followed by Capmul MCM (MC MG), Captex 1000 (MC TG) and olive oil (LC TG), i.e. $\text{Peceol} \geq \text{Capmul MCM} \geq \text{Captex 1000} = \text{olive oil}$. The LC MG suspensions showed a significantly higher AUC than the TG suspensions ($p \leq 0.05$) and the MC MG suspension showed a significant higher AUC than the LC TG suspension, whereas there was no statistically significant difference between the other lipid suspensions (Table II). Additionally, a trend towards increased t_{\max} was observed in cases where lipid excipients were used indicating that solubilizing benefits of the lipids were time-delayed.

The aqueous nilotinib suspension in the fasted state led to an AUC of 14369 ± 3747 ng.h/mL. In the cases of the lipid suspensions, the highest overall AUC was observed for the LC MG (AUC of 13103 ± 2557 ng.h/mL) and MC MG (AUC of 11210 ± 5476 ng.h/mL), which were not statistically dissimilar from the aqueous suspension. Critically however, no bioavailability enhancement was evident for any of the lipid suspension. On the contrary the MC TG showed a significant 2.8-fold decrease and the LC TG a significant 4.0-fold decrease ($p < 0.01$). Thus, relative to the aqueous suspension in the fasted state both TG formulations showed a significant reduced bioavailability.

Dosing nilotinib to rats with free access to food resulted in a similar AUC compared to the dosing in the fasted rats. Thus, the profound food effect observed in humans was not evident in the employed rat model. It is notable that the MRT was prolonged in the fed state study, with the MRT being comparable to the MC suspension study group (Figure 4 B). A MRT performance ranking of aqueous suspension (fasted) = $\text{Peceol (LC MG)} \leq \text{olive oil (LC TG)} \leq \text{Captex 1000 (MC TG)} = \text{Capmul MCM (MC MG)} = \text{Aqueous suspension (fed)}$ was observed.

405 **Table II** Pharmacokinetic parameters of nilotinib after oral administration of 20mg/kg to male sprague-dawley rats (n = 5). t_{\max} and MRT given
 406 as median (range), all other parameters as mean \pm SD.

Pharmacokinetic parameters						
	Aq. suspension Fasted	Aq. suspension Fed	MC TG (Captex 1000)	LC TG (olive oil)	MC MG (Capmul MCM)	LC MG (Peceol)
c_{\max} [ng/mL]	2648 \pm 676	1423 \pm 321	774 \pm 345	605 \pm 550	1321 \pm 1038	2801 \pm 756
t_{\max} [h] (range)	2 (2-4)	2 (2-4)	6 (4-10)	4 (2-6)	4 (1-6)	4 (4-6)
AUC 0-10 h [ng*h/mL]	13984 \pm 3576	9350 \pm 2431	4264 \pm 1949	3151 \pm 2516	8323 \pm 4873	12393 \pm 2666
AUC 0-inf. h [ng*h/mL]	14369 \pm 3747	13335 \pm 3487	5168 \pm 2197	3548 \pm 2711	11210 \pm 5476	13103 \pm 2557
MRT [h] (range)	4.17 (3.32-4.81)	7.79 (7.49-9.60)	7.37 (5.91-8.04)	6.18 (4.67-7.70)	8.35 (6.94-8.64)	5.57 (5.05-7.12)
F_{rel} [%] ^a	100	92.80 \pm 24.27	35.96 \pm 15.29	24.69 \pm 18.87	78.01 \pm 38.12	91.19 \pm 17.80

407 ^a Relative to the aqueous suspension in the fasted state

3.3 Drug Solubilization during *in vitro* dispersion and digestion.

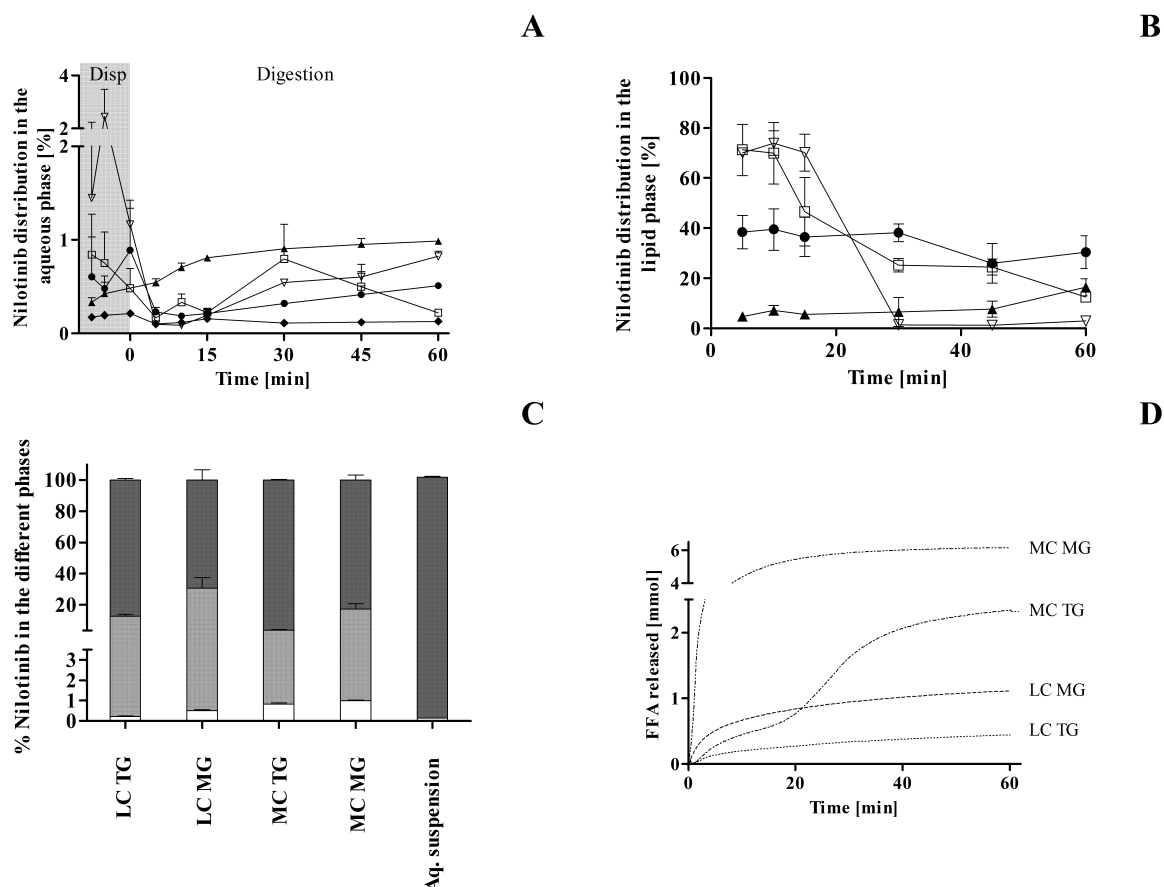


Figure 5 Results of the *in vitro* lipolysis (mean \pm SEM; n=3) for the aqueous suspension (\blacklozenge), LC MG [Peceol] (\bullet), LC TG [olive oil] (\square), MC MG [Capmul MCM] (\blacktriangle), MC TG [Captex 1000] (∇). A: Percent of nilotinib in the aqueous phase, B: Percent of nilotinib in the calculated lipid phase, C: Distribution of nilotinib across the aqueous (white), calculated lipid (light grey) and solid phase (dark grey) after 60 min of digestion, D: Free fatty acids released over time for the studied lipid-based formulations corrected for a blank during 60 min of digestion.

In order to provide an improved mechanistic understanding of the *in vivo* pharmacokinetics, further *in vitro* studies were undertaken. Thus, the lipid suspensions were assessed using the dynamic *in vitro* lipolysis model. Lipid suspensions were dispersed initially in biorelevant buffer representing the fasted state for 10 min prior to initiation of the digestion by the addition

of lipase. The release of nilotinib into the aqueous phase during the dispersion and digestion is shown in Figure 5 A.

Overall for the four lipid suspensions, the extent of drug solubilised in the aqueous phase was higher compared to the aqueous suspension throughout dispersion and digestion. However, the percent of the dose solubilised in the aqueous phase was low at between 0.1 and 1.1 % of the dose. The rank order of the five tested formulations was Capmul MCM (MC MG) = Captex 1000 (MC TG) > Peceol (LC MG) > olive oil (LC TG) = aqueous suspension. Upon dispersion of the lipid suspensions the highest concentration in the aqueous phase was observed for the MC TG suspension with 2.5 ± 1.8 % of the dose solubilised, whereas at the end of digestion the highest nilotinib concentration of 1.0 ± 0.1 % was observed for the MC MG formulation. It was notable that upon the start of digestion the initial solubilisation capacity for nilotinib was reduced for the MC TG, LC TG and LC MG suspensions. However, for these three lipid suspensions an increase of the nilotinib concentration was observed after the initial drop, indicating that the post digestive products aid the solubilisation of nilotinib. In the case of the MC MG suspension the nilotinib concentration in the aqueous phase steadily increased during dispersion and digestion. These observations translated to a SLAD from 0.16 for the aqueous suspension to 0.28– 1.09 for the lipid suspensions after 60 min of digestion.

Figure 5 C presents the distribution of drug between the aqueous phase, pellet phase and ‘oil’ phase after 60 min of digestion of the sample. As expected most of nilotinib was recovered in the solid phase for all five suspension formulations, which mainly reflects suspended drug particles. In the case of the poorly dispersible TG suspensions, an oily lipid phase was particularly evident during the initial stages of digestion. This oily phase most likely reflected undispersed and undigested or partially digested lipids in the formulation. The quantity of drug

in this phase was theoretically calculated using a mass balance approach i.e. by subtracting the quantity of drug determined analytical in the pellet and aqueous phase samples from the total amount of drug present. Interestingly, at the initial phase of digestion, a greater amount of drug was calculated to be within this oil phase for the TG formulations relative to the MG formulations (Figure 5 B). Up to 85 % and 83 % of nilotinib's dose was theoretically calculated to reside within this oil phase on top of the media in the lipolysis vessel for the olive oil (LC TG) and Captex 1000 (MC TG) formulations, respectively. These amounts exceeded the equilibrium solubility of the drug within these oils significantly indicating that nilotinib was likely to be present as suspended drug crystals within this phase. By comparison, for the MG formulations much lower amounts of drug were present in this initial phase of digestion, with 52 % and 10 % in Peceol (LC MG) and Capmul MCM (MC MG), respectively. Therefore, it would appear that the formulations that performed poorest *in vivo* displayed the greatest amount of drug within this oil phase in the initial phase of digestion. As digestion proceeded, the amount of drug within this oil phase decreased, most likely reflecting the digestion of these lipid formulations, which was mirrored by the increase in free fatty acids (FFA) released (Figure 5 D). In particular, Captex 1000 (MC TG) distinct increase in FFA generated between 15-30 min of digestion corresponded to the decrease in nilotinib concentrations in the oil phase from 70.2 ± 12.7 % at 15 min to 1.4 ± 1.0 % at 30 min. A similar, albeit less dramatic, decrease in the amount of drug estimated in the oil phase was observed for the olive oil formulation between 15 to 30 min. However, the overall extent of digestion for the olive oil (LC TG) suspension was lower relative to the other formulations. Following completion of a back titration to pH 9 to adjust for the non-ionised FFA the rank order of digestibility was olive oil (1.30 mM FFA released) < Peceol (2.22 mM FFA released) \leq Captex 1000 (2.93 mM FFA released) < Capmul MCM (6.27 mM FFA released).

3.4 Wettability of nilotinib crystals with lipid and aqueous media

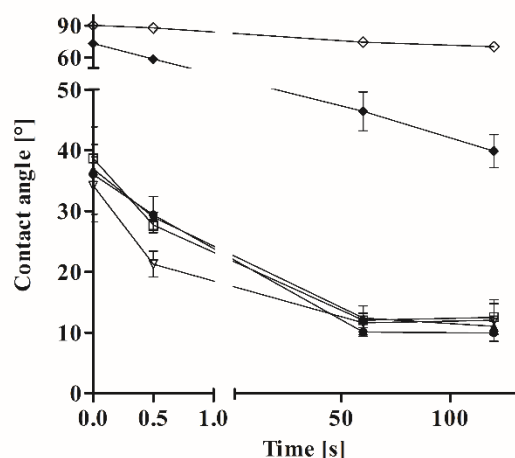


Figure 6 Wettability of nilotinib by water (◇), 0.5% methyl cellulose solution (◆), Peceol (●), olive oil (□), Capmul MCM (▲), Captex 1000 (▽). Measurements for each time point are done on 3 disks ($n \leq 5$).

In order to probe whether differing wetting characteristics of nilotinib crystals between the various formulations could be used to explain lower bioavailability of the TG formulations, the wettability of nilotinib by the five formulation vehicles and pure water was determined utilizing the sessile drop technique. The results are presented in Figure 6. The equilibrium contact angle was reached after 60 seconds of the measurement. Water was used as a reference which confirmed the hydrophobic nature of nilotinib with contact angles of up to 80° . Additionally, the four lipid excipients and the 0.5% methyl cellulose vehicles from the *in vivo* study were tested. It was observed that all lipid vehicles used in this study wetted nilotinib better than the aqueous 0.5% methyl cellulose with contact angles between 10.1° and 12.5° . It was further observed that the lipids penetrated the nilotinib disk much faster covering the nilotinib crystals in a lipid film.

4 Discussion

Lipid excipients have shown great potential to enhance oral bioavailability by increasing solubilisation in intestinal fluids and improving intestinal permeability/uptake (15). Lipid suspensions have been investigated for a number of drugs as an approach to enhance *in vivo* bioavailability with the overall experience that in the majority of reported studies a benefit was observed (19-23). However, the utility of any LBF as a bio-enabling strategy is highly dependent on the molecular properties of the candidate drug, and in particular both hydrophobic and lipophilic properties of the drug. In the case of highly hydrophobic 'brick dust' molecules, there are knowledge gaps in the literature on the usefulness of lipid suspensions and there is a need for more studies involving high T_m and high $\log P$ drugs to assess potential *in vivo* merits.

Nilotinib is a hydrophobic compound (T_m of 236 °C), but it is also highly lipophilic ($\log P$ 4.95) and displays an 82 % higher bioavailability on administration with a fat rich meal (33). In this study, the solubility of nilotinib in lipids was found to be very low. While solubility increased in more polar oils such as MC and MG, the overall solubility in lipids was insufficient to solubilise the dose. Solubility screening in biorelevant media confirmed a higher solubility in the fed state intestinal fluids with a FeSSIF/FaSSIF ratio of approximately 10. However, overall with a SLAD of 0.16 in FaSSIF, oral absorption of nilotinib is clearly solubility limited. Subsequent biorelevant solubility screening that mimicked the post digestive state appeared to lead to further enhancements in *in vivo* solubilisation, which may in part explain the increased bioavailability observed clinically in humans in the fed state. It might therefore have been anticipated that the *in vivo* study would show increased bioavailability in the fed state as well as an increased exposure following dosing as lipid suspensions. However, in the employed rat

model, a food effect was not observed most likely reflecting limitations of this model, which are described in further detail below. Additionally, despite the above *in vitro* results the lipid suspensions did not show an increased exposure compared to the aqueous suspension.

The *in vivo* study results showed that both MG and TG suspension did not result in a bioavailability enhancement. Both MG suspensions were comparable to the aqueous suspension and a trend towards a better performance of the MG suspensions compared to the TG suspensions was observed. While only the LC MG suspension was statistically significant different from both TG suspensions ($p < 0.01$), the MC MG suspension was significantly different from the LC TG suspension ($p < 0.05$). In the case of the TG suspensions, bioavailability was significantly lower relative to the LC MG and aqueous suspension ($p < 0.01$), respectively. While the previous biorelevant dispersion experiments suggested a higher solubilisation for the MC formulations relative to the LC formulation, this performance was not evident in the *in vivo* study in case of the MGs. The better performance of the Peceol (LC MG) versus Capmul MCM (MC MG) suspension may reflect other effects of LC versus MC lipids on intestinal uptake and/or absorption. While the impact of MC and LC remains unclear, it appears that the long chain digestion products more readily maintain solvation capacity (36). Additionally, it was interesting to note that the MRT of LC MG was significantly lower compared to the MRT of MC MG (i.e. 5.57 h versus 8.35 h) indicative of a faster absorption process for the LC MG suspension ($p < 0.01$). In general, MRT is an indicator of the average time a drug molecule spends in the body. As none of the excipients used in this study are known to significantly alter distribution, metabolism or excretion, an increased MRT between study groups is indicative of a delayed absorption phase, most likely reflecting prolonged drug residence time in the GIT.

537 Further *in vitro* experiments were conducted to provide mechanistic insights into the results
538 obtained *in vivo*. The formulations were therefore tested in an *in vitro* digestion and dispersion
539 experiment to explore the changes in solvents solvation capacity over time as possible causes
540 for a lower dissolution rate and bioavailability of the lipid formulations (Figure 5). The
541 dissolution/release into the aqueous phase was limited in all tested formulations (Figure 5 A).
542 Both MC formulations and the LC MG formulations performed better than the aqueous
543 suspension. The drug amount in the aqueous phase after 60 min of digestion was influenced by
544 the excipients chain length with MC excipients resulting in higher concentrations than LC
545 excipients. However, the performance ranking observed based on drug concentrations in the
546 aqueous phase after 60 minutes digestion, did not match the *in vivo* performance ranking. In
547 fact, *in vivo* the aqueous suspension showed similar or higher bioavailability compared to the
548 lipid suspensions. This suggests that the amount of drug in the aqueous phase may not be a
549 strong predictor of lipid suspension performance, but rather other factors that govern drug
550 dissolution and release from lipid suspensions may be relevant such as limited solubility in
551 lipids, drug-excipient interactions and crystalline particle characteristics. While the aqueous
552 phase data seemed to overestimate the *in vivo* performance of lipid suspensions, it is also
553 possible that the test setup underestimated the aqueous suspension. The saturation levels in
554 such closed *in vitro* test settings are quickly reached for low soluble drugs limiting the
555 dissolution and release into the aqueous phase. Clearly, the presence of digestible lipid
556 excipients increased nilotinib's solubilisation post digestion as shown by the solubility studies
557 in the artificial post-digestive media. However, it is not clear whether the enhanced
558 solubilisation will lead to increased absorption or whether release and/or dissolution of drug
559 crystals is the rate limiting step to absorption. An additional absorption step would allow further
560 insights and the evaluation of the release and dissolution rate for such low soluble compounds
561 like nilotinib (27, 37). Therefore, it may also reflect the limitations of the standard *in vitro*

lipolysis test (37, 38). Furthermore, nilotinib is a weak base that showed increasing solubility with decreasing pH of the media. The better solubility in a gastric media may generate higher initial concentrations in the intestine leading to a better absorption. A two-step gastro intestinal lipolysis may be beneficial for weakly basic compounds, like nilotinib, to get a better match with the *in vivo* data (39).

While most of nilotinib was recovered in the solid phase (Figure 5 C), it appeared that for the TG-based suspensions nilotinib concentrations in the lipid phase remained high in the initial stages of digestion. At the start of digestion approx. 70 % of the nilotinib dose resided within the lipid TG phase in the vessel decreasing to approx. 20 % at the end of digestion (Figure 5 B). This was equivalent to approx. 1244-fold and 165-fold excess of equilibrium drug solubility in the olive oil and Captex 1000 phase, respectively. Such high amounts of drug within the TGs indicated that nilotinib crystals remained unreleased within the oil phase on top of the vessel and were not sampled (the samples were taken from the middle of the vessel) and consequently not recovered in the solid pellet phase that was collected after ultra-centrifugation. In fact, for the TG suspensions, a distinct undispersed “oil” phase was evident at the top of the vessel on dispersion and during the initial phases of digestion. As samples were collected from the middle of the vessel, drug crystals that were retained within the oil phase were not sampled. Consequently, the major reason for the higher amount of drug in the oil phase was the poor dispersibility of the TG based suspensions. This behaviour of nilotinib indicated a pronounced hydrophobic interaction between the TG excipients and nilotinib crystals, potentially delaying the release of nilotinib crystals into aqueous media which may lead to slower overall dissolution. Such a kinetic effect was likely of relevance for the *in vivo* performance of the formulations. In the case of the MG excipients that displayed greater dispersibility in the biorelevant media relative to TGs, only a minor (LC MG) or no (MC MG) lipid layer was

evident in the vessel. The overall amount of drug estimated to reside within the lipid layer for these MG formulations was lower than the TG formulations and was not influenced to any great extent by digestion. Collectively, these observations may explain the lower bioavailability observed for the TG formulations relative to the MG formulations, where nilotinib crystals were not released from the undispersed oil phase leading to a delayed release of nilotinib crystals within the intestine which may have reduced absorption overall.

The propensity for nilotinib to be released and to dissolve in any solvent is fundamentally determined by the balance between the crystal lattice energy and the interactions with the solvent. Additionally, the rate of dissolution depends on a number of factors including particle size, viscosity of the solvent, hydrodynamics, the overall available volume of solvent, the solvents solvation capacity and wettability of the crystalline particles (40). When comparing the lipid and aqueous suspensions used *in vivo* in this study, all of these factors were expected to remain constant except for wettability, as the addition of lipids alters the effective wettability of nilotinib crystals. For all lipid excipients a low contact angle of 10.1 ° - 12.5 ° was observed, whereas for 0.5% methyl cellulose, a higher contact angle of 46.4 ° was obtained. This indicated a stronger interaction between nilotinib crystals and the lipid vehicles compared to the aqueous vehicle. Thus, greater wettability of the nilotinib crystals within the lipid suspensions may have been a contributor to the *in vivo* performance. The observations of greater wettability in lipids further supported the slower release of crystals from the undispersed oil layer observed in the *in vitro* lipolysis test. Therefore, we suggest that the pronounced hydrophobic interactions between the lipids and nilotinib crystal will favour the formation of a lipid film around the nilotinib crystals, which remains intact even after dispersion in aqueous media and during the initial phases of digestion. This surface bound lipid film may delay wetting of nilotinib crystals by aqueous media and therefore result in delayed

dissolution of the drug. In addition, the partition of drug through the lipid layer into the aqueous media will be limited by the inherent low solubility of this drug within lipids. Over time the lipid film will gradually be removed, either via dispersion of the lipid excipient into the aqueous media or digestion of the lipids, leading to complete wetting of the drug crystals by the aqueous media and dissolution of the crystals in aqueous media can proceed. A delayed release from the formulation would also explain the significant exposure decrease for the TG formulations. The delayed release from lipid suspensions was further supported by observations from the *in vivo* mean residence time (MRT). The MRT was significantly longer for the LC TG, MC TG and MC MG formulations, relative to the aqueous suspensions ($p < 0.05$). It therefore seems as if nilotinib dissolution and absorption from the TG suspensions, and to a lesser extent MC MG suspension, was slower and may have led to a reduction on bioavailability for the TG formulations. Overall, a performance increase might be achieved by preventing the lipid film formation utilizing for example a chase dosing regimen, in which the lipid and drug are administered consecutively (14) or in a capsule-in-capsule approach. Additionally, the use of nilotinib with more polar and amphiphilic excipients such as surfactants may result in a better performance.

While the study demonstrated that rats did not show a food effect for nilotinib in this specific model, this may be related to the model itself or species-specific physiological differences, and in particular differences regarding bile secretion and/or gastro-intestinal volumes (41). One limitation of the fed state study leg employed in this study is that the rats had free access to food throughout the experimental procedure. As a result, this may have led to variability in the amount of food present in the stomach and intestine of rats. While it has been demonstrated that Sprague-Dawley rats can be used to predict food effects in humans for several drugs, it is recommended to use a specific protocol involving a homogenized FDA breakfast (42).

However, due to logistical constraints an FDA style fed state protocol was not employed in this study. Nevertheless, it was noteworthy that the MRT in the fed state was significantly longer than the MRT in the fasted state, which might reflect a delayed absorption process in the fed state. Interestingly, relative to the aqueous suspension in the fasted state, the MC MG, MC TG and LC TG suspensions displayed a longer MRT, and comparable to that obtained for the aqueous suspension in the fed state. This may indicate that these lipid excipients mimicked fed state conditions in terms of a slower absorption, due to the digestion process (43).

5 Conclusion

This study focused on providing new *in vivo* and *in vitro* insights for the ‘brick dust’ drug nilotinib in LBFs. *In vivo* neither TG nor MG lipid suspension resulted in an increase in bioavailability of nilotinib relative to an aqueous suspension. Nevertheless, it was demonstrated that dispersibility of the lipids was a major contributing factor to the performance of nilotinib lipid suspensions. A higher *in vivo* exposure was observed for nilotinib lipid suspensions based on MG compared to TG lipids. The poorly dispersible TG suspensions resulted in a significantly reduced bioavailability compared to the aqueous suspension. Subsequent, *in vitro* studies suggested that the lower bioavailability observed for both TG suspensions most likely reflected a slower release of nilotinib from these formulations which were also reflected by a slower *in vivo* absorption. The key determinants for the success of a lipid suspension using a ‘brick dust’ molecule appeared to be the dispersibility and release from the lipid excipient.

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